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Removal of chromium(VI) and cadmium(II) from aqueous solution by a bacterial biofilm supported on granular activated carbon

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Abstract

A biofilm of *Arthrobacter viscosus*, supported on granular activated carbon, removed between 100% and 50% of Cr(VI) and between 100% and 20% of Cd(II) from solutions with initial concentrations between 4–11 mg_{metal} l^{-1} and a flow residence time of 1.2 min. For experiments of lower initial concentrations, a steady-state removal of 50% was reached after 71 bed volumes of Cr solution passed through the biosorbent bed and a steady-state removal of 30% was reached after 47 bed volumes of Cd solution passed through a similar bed. Final uptakes of 8.5 mg_{Cr} g^{-1}_{carbon} and 4.2 mg_{cd} g^{-1}_{carbon} were determined for initial concentrations of 10 mg_{Cr} l^{-1} and 11 mg_{Cd} l^{-1}, respectively. The influence on the overall process of two different surface treatments of the support was evaluated and compared with the behavior of a support not treated.

Introduction

The increasing concern with environmental pollution significantly motivates the investigation and development of safe technologies. The retention of contaminants by a biofilm supported on granular activated carbon is one of the promising technologies. The use of bacteria as biosorbents is a fast growing field in remediation due to their small size, their ubiquity, their ability to grow under controlled conditions and their resilience to a wide range of environmental situations (Urrutia 1997). Biosorption is the accumulation of metals without active uptake and can be considered as a collective term for a number of passive accumulation processes which may include ion exchange, coordination, complexation, chelation, adsorption and microprecipitation (Duncan *et al.* 1994). Other authors (Woodburn *et al*. 1999) reported that biosorption utilizes the ability of biological materials to accumulate heavy metals from waste streams either by metabolical mediation or by purely physico-chemical pathways of uptake. The use of a biosorption system consisting of a biofilm supported on granular activated carbon combines the ability of the biofilm to remove heavy metals with the ability of the activated carbon to remove organic compounds (Scott & Karanjkar 1995).

Arthrobacter viscosus is a good exopolysaccharide producer which, by itself, would allow to foresee good qualities for support adhesion and for metal ions entrapment (Scott & Palmer 1988). The use of activated carbon as a support is justified by the fact that this material is a versatile adsorbent due to its high surface area, porous structure, high adsorption capacity and surface chemical nature, which can be appropriately modified by physical and chemical treatments to enhance the extent of a given adsorption process (Radovic & Rodrígues-Reinoso 1997).

Among different heavy metals that may be removed from liquid solutions by biosorption, chromium demands special attention as it may present several oxidation states. In acid solution, the hexavalent form, CrO_4^{2-}, constitutes a strong oxidizing agent easily reduced to the trivalent form which precipitates as $Cr(OH)_3$, while in basic pH conditions Cr^{3+} is oxidized to the hexavalent form (Katz & Salem 1994). Besides, a possible reduction of CrO_4^{2-} to Cr^{3+} may be performed by the biofilm itself, after penetration into the cells. This metabolic reduction has already

been studied and modeled for different pure bacterial cultures (Wang & Cheng 1997).

The present study involves the investigation and development of an innovative process for the removal of two heavy metals, with different behavior in solution: chromium that is present in the anionic form and cadmium in the cationic form. The effects of the initial concentration and surface treatment of carbons were tested.

Material and methods

Materials

Arthrobacter viscosus was obtained from the Spanish Type Culture Collection of the University of Valência. Aqueous chromium and cadmium solutions were prepared by diluting K_2CrO_7 and $CdSO_4 \cdot 8/3H_2O$ in distillated water. All glassware used for experimental purposes was washed in 60% (v/v) nitric acid and subsequently rinsed with deionised water to remove any possible interference by other metals. Atomic absorption spectrometric standards were prepared from 1000 $mg_{Cr}\ l^{-1}$ and 1000 $mg_{Cd}\ l^{-1}$ solution. The support was characterized by N_2 adsorption (77K), with an ASAP Micromeritics 2001, in order to evaluate surface area, pore size distribution and pore volume. Surface treatments consisted in washing the granular activated carbon (GAC) for 1 h at 90 °C, with 1 M HNO_3 or 1 M H_2O_2, in order to develop different surface groups in the carbon surface, after thermal treatment in N_2 atmosphere. Surface functional groups identification was carried out by Boehm titration, FTIR, TPD and XPS. Universidade Nova de Lisboa, Departamento de Química, made the structure and surface characterization as well as surface treatment of carbons.

Methods

All experimental work was conducted in duplicate. GAC was placed in a 250 ml Erlenmeyer flask to which 150 ml distilled water was added. It was sterilized at 120 °C for 20 min to release the air inside the pores. Then it was placed in column. Minicolumns (internal diameter = 0.9 cm, ht = 30 cm) were used for open systems studies, partially filled with GAC (6 g) with a Langmuir area of 1270 $m^2\ g^{-1}$ and an average pore diameter of 2 nm. The microorganism culture and the nutrient broth were pumped through the bed aiming the formation of the biofilm. Two different media, with different concentrations of peptone, were used to grow the microorganism for 3 d, aiming the optimization of the adhesion. The formation of the biofilm was observable by naked eye. After this period of time the bed was washed out and the metal solutions were passed through the column with a flow rate of 10 ml min^{-1}. Samples (5 ml) were taken, centrifuged and analyzed for metals using atomic absorption spectrophotometry, AAS. The results were expressed as removal percentage. At the end of each run the column was washed out and samples of the effluent were seeded in Petri plates with nutrient agar to assess the metabolic activity of the microorganism.

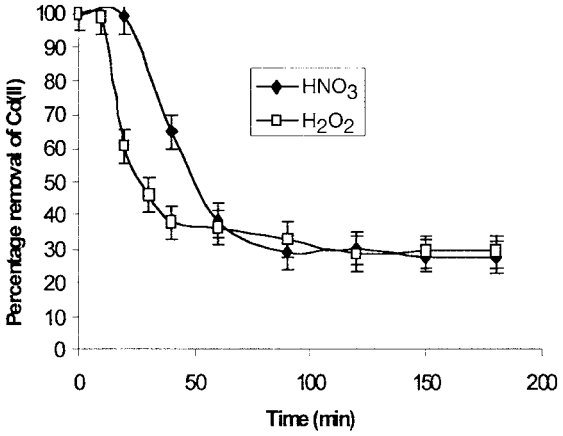

Fig. 1. Removal of cadmium by a biofilm of *Arthrobacter viscosus* supported on granular activated carbon, with surface treated with HNO_3 and with H_2O_2. The initial concentration of chromium was 5 mg l^{-1}, with a flow rate of 10 ml min^{-1} and a residence time of 1.2 min. Steady-state 30% removal reached after 47 bed volumes of solution passed through the mini-column.

Results and discussion

The surface treatments applied to the support led to two different surfaces as confirmed by the techniques referred above. The one treated with nitric acid presented more surface functional groups with oxygen than the one treated with oxygen peroxide. These groups were not detected in GAC surface without any treatment, used as control. Both treatments led to a smaller average pore radius, compared to control.

The removal experiments, all of them with a flow residence time of 1.2 min, revealed different behaviours between the two metals. A steady-state removal of 30% was reached after 47 bed volumes of Cd solution, with 5 mg l^{-1}, passed through the biosorbent

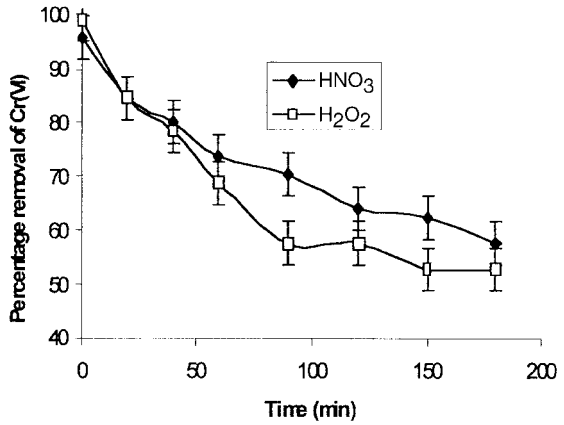

Fig. 2. Removal of chromium by a biofilm of *Arthrobacter viscosus* supported on granular activated carbon, with surface treated with HNO_3 and with H_2O_2. The initial concentration of chromium was 4 mg l^{-1}, with a flow rate of 10 ml min^{-1} and a residence time of 1.2 min. Steady-state 50% removal reached after 71 bed volumes of solution passed through the mini-column.

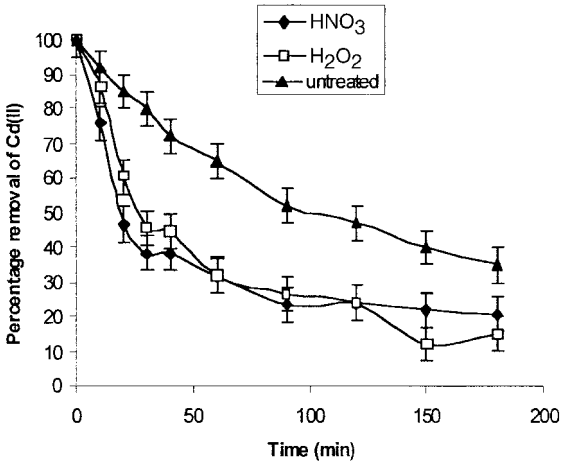

Fig. 3. Removal of cadmium by a biofilm of *Arthrobacter viscosus* supported on granular activated carbon, with surface treated with HNO_3, with H_2O_2 and without treatment for comparison. The initial concentration of chromium was 11 mg l^{-1}, with a flow rate of 10 ml min^{-1} and a residence time of 1.2 min.

bed (Figure 1). In steady-state conditions, no difference between the two supports is detected, the same happening at higher initial concentrations (Figure 3). A steady-state removal of 50% was reached after 71 bed volumes of Cr solution, with 4 mg l^{-1}, passed through the column, with a support treated with H_2O_2 (Figure 2). When the support is treated with HNO_3 the steady-state removal increases 10%. At a higher concentration of Cr, that is 10 mg_{Cr} l^{-1}, a better removal is attained with the H_2O_2 treated support (Figure 4), with a steady-state removal of 70%. Final uptakes of 8.5 mg_{Cr} g^{-1}_{carbon} and 4.2 mg_{Cd} g^{-1}_{carbon} were determined, respectively, for initial concentrations of 10 mg_{Cr} l^{-1} and 11 mg_{Cd} l^{-1}.

The removal of both metals was fast and presented a typical biosorption kinetics, which includes two phases: the first one is associated with the external cell surface, biosorption itself, and the second one is an intra-cellular accumulation/reaction, depending on the cellular metabolism (Tavares *et al.* 1995). There are some evident differences between the removal processes of the two metals: Cd is present as Cd^{2+} and has a strong xenobiotic effect on the biofilm. Its fixation may occur on the polysaccharide net, but mainly on GAC, as the process seems to be dependent on the support pore size distribution, which is altered as the support is submitted to any surface treatment. The saturation of the binding sites will, eventually, lead to 0% removal.

On the other hand, Cr is in an anionic state as a strong oxidizing agent. Its fixation occurs mainly on the biofilm surface, as its relatively big anionic radius would not allow direct adsorption on GAC surface. Eventual reduction to Cr^{3+} must occur metabolically (Katz *et al.* 1994), that is accumulation and reaction inside the cells, as no precipitate was detected inside or at the outlet of the column, as observed by centrifugation of samples to be analyzed for safety of equipment. The effluent of the column always kept the characteristic yellow color of the hexavalent form. Besides, the quantification method used in this work, AAS, detects the total amount of metal, no matter the oxidation state it is in.

Effect of initial concentrations of metals

The steady-state removal percentage seems to decrease with increasing initial metal concentration. The removal of Cd reaches 30% with an initial concentration of 5 mg_{Cd} l^{-1}, which is reduced to 20% when initial metal concentration rises to 11 mg_{Cd} l^{-1} (Figures 1 and 3). On the other hand, the steady-state removal of Cr reaches 60% with an initial concentration of 4 mg_{Cr} l^{-1}, while an initial concentration of 10 mg_{Cr} l^{-1}, leads to a steady-state removal of 50%, for the same kind of support, HNO_3 treatment (Figures 2 and 4). This can be explained by the saturation of the binding sites of the biological matrix. The differences in retention mechanism between the two metals are underlined by the two values of uptake, that is 8.5 mg_{Cr} g^{-1}_{carbon} and 4.2 mg_{Cd} g^{-1}_{carbon}, considering the difference in atomic weigh between Cr and Cd.

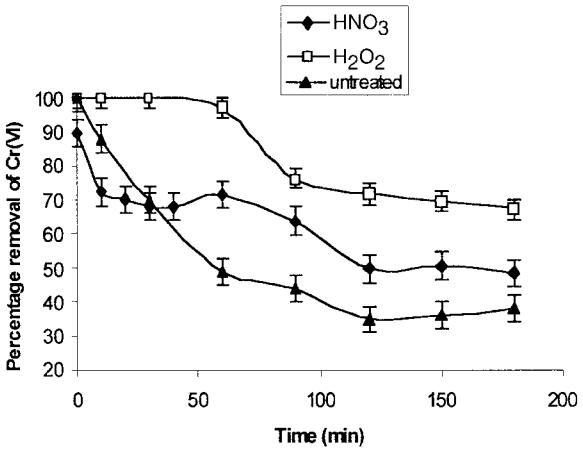

Fig. 4. Removal of chromium by a biofilm of *Arthrobacter viscosus* supported on granular activated carbon, with surface treated with HNO_3, with H_2O_2 and without treatment for comparison. The initial concentration of chromium was 10 mg l^{-1}, with a flow rate of 10 ml min^{-1} and a residence time of 1.2 min.

Effect of the surface treatment of carbons

The surface treatments seem to have different effects on the removal of the two metals. There is no difference between the two supports in the removal of Cd, particularly after 40 bed volumes of solution passed through the beds (Figure 1). Similar behavior is detected for higher initial concentration of the metal (Figure 3). Actually, a better performance is detected with the control support. Possibily, this is due to the fact that retention of Cd occurs mainly in GAC, not in the biofilm as a consequence of its high xenobiotic character. Being a cation, its relative smaller radius allows the approach to the GAC surface. It was observed that the average pore radius is bigger in untreated GAC than in treated ones, which may allow a more effective adsorption, directly on the support.

The retention of Cr has different characteristics, occurring mainly on the biofilm. There is an advantage in treating the support surface (Figure 4), and the distinction between the two treatments is more evident approaching the steady-state removal (Figure 2). The best results obtained with GAC treated with HNO_3 could be connected with the stronger presence of acidic groups in the surface of this kind of GAC. These acidic groups contribute to the adhesion of the biofilm and consequently to the best removal (Avery & Tobin 1992). At higher Cr initial concentrations, with a stronger driving-force to approach the surface, with or without biofilm, a better removal is attained with the H_2O_2 treated support. A higher content of surface functional groups with oxygen may be responsible for the fixation of CrO_4^{2-} directly on the support.

Conclusions

A biofilm of *Arthrobacter viscosus* supported on granular activated carbon is able to remove chromium and cadmium from dilute solutions and can be applied in wastewater remediation. The different oxidation states of the ions under study lead to different mechanisms of retention. While Cd^{2+}, with an evident xenobiotic effect on the biofilm, is probably adsorbed by the support itself, being sensitive to its pore structure, Cr^{6+} is mainly entrapped by the exopolisaccharide net and this is enhanced by previous surface treatment of the support. The promising results with this ion indicate a possible application of an adsorption-reaction mechanism model with biosorption followed by a metabolic reduction of the hexavalent form that will be verified by long-term experiments. These preliminary studies give no indication of Cr^{6+} reduction outside the biofilm.

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References

Avery S, Tobin J (1992) Mechanisms of strontium uptake by laboratory and brewing strains of *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* **58**: 3883–3889.

Duncan JR, Brady D, Stoll A (1994) Biosorption of heavy metals cations by non-viable yeast biomass. *Environ. Technol.* **15**: 429–438.

Fourest E, Roux JC (1992) Heavy metal biosorption by fungal mycelial by-products: mechanisms and influence of pH. *Appl. Microbiol. Biotechnol.* **37**: 399–403.

Katz S, Salem H (1994) *The Biological and Environmental Chemistry of Chromium*. New York: VCH Publishers.

Radovic LR, Rodrígues-Reinoso F (1997) Carbon materials in catalysis. In: Throweir PA, ed., *Chemistry and Physics Carbon*, Vol. 25. New York: Marcel Dekker, pp. 243–358.

Scott A, Karanjkar A (1995) Adsorption isotherms and diffusion coefficients for metal biosorbed by biofilm coated granular activated carbon. *Biotechnol. Lett.* **17**: 1267–1270.

Scott A, Palmer SJ (1988) Cadmium bio-sorption by bacterial exopolysaccharide. *Biotechnol. Lett*. **10**: 21–24.

Tavares MT, Martins C, Neto P (1995) Biotreatment of Cr (VI) effluents. In: Sengupta, AK, ed., *Hazardous and Industrial Wastes*. Lancaster: Tecnomics Publishing Co., pp. 223–232.

Urrutia MM (1997) General bacterial sorption processes. In: Wase J, Forster C, eds., *Biosorbent for Metal Ions*. London: Taylor and Francis Publishers, pp. 39–66.

Wang YT, Shen H (1997) Modelling Cr(VI) reduction by pure bacterial cultures. *Water Res.* **7**: 727–732.

Woodburn, GM, Yu, Q, Matheickal, JT (1999) Biosorption of cadmium(II) from aqueous solutions by pre-treated biomass of marine alga *Durvillaea potatorum*. *Water Res*. **32**: 400–406.